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Does dexamethasone interfere with process of blastocyst implantation in the pregnant rat uterus?

هل يتعارض الدكساميثازون مع عملية انغراس الكيس الاروميي في رحم الجرذان الحوامل؟

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Abstract

Results has been revealed that the blastocysts from both the control and treated groups were successfully implanted. The decidual tissue as one of the indicators of success of implantation was formed .Implantation of blastocysts started in the antimesometrial side of uterus at day 7 of pregnancy , then the position of the embryos were shifted to mesometrial side of the uterus. Conclusion of this study have shown that dexamethasone is safe during implantation period, but probable side effects might occur during subsequent stages of pregnancy.

Key words: Rats, dexamethasone, implantation and decidual tissue

الخلاصة

أظهرت النتائج ان الاكياس الارومية تمكنت من الانغراس بنجاح وتم تكوين النسيج الساقطي في ارحام الجرذان من كلا مجموعتي السيطرة والعلاج. اعطت نتائج اليوم السابع من الحمل لمجموعتي السيطرة والمعالجة نفس النتائج من خلال حصول الانغراس وتكوين النسيج الساقطي في بطانة الرحم اولا في المنطقة المضادة للمساريق الرحمي والذي يعتبر مؤشر لنجاح الانغراس. اما اليوم العاشر كانت مواقع الانغراس واضحة جدا وان الجنين في هذه المرحلة قد تحول من المنطقة المضادة للمساريق الرحمي الى منطقة المساريق الرحمي واما النسيج الساقطي فكان باعلى درجة من النمو والتطور . نستنتج من هذه الدراسة ان استعمال الديكساميثازون يعد آمنا اثناء فترة الانغراس. ولكن قد تكون له آثار سلبية والتي من المتوقع أن تحصل في فترات لاحقة من الحمل .

Introduction:

Dexamethasone (DX) is considered as a synthetic form of a long acting glucocorticoid (GC) with a potency as steroidal anti-inflammatory drug[(1)]. The biological half-life is about 3 hours[(2)], although the duration of action may be much longer [(3)]. Dexamethasone is listed as a pregnancy category C drug [(4)].Clinically it has been used in perioperative setting, including prophylaxis against postoperative nausea and vomiting, reduction of airway and cerebral oedema [(5)]. Dexamethasone is used for prevention and treatment of chemotherapy related and postoperative nausea and vomiting [(6)]. DX treatment is used for prevention of the respiratory distress syndrome in premature infants [(7)]. The most widespread use of glucocorticoids is in asthma and inhaled glucocorticoids have revolutionized treatment and now become the mainstay of therapy for patients with chronic disease[(8,9)] .Common indications for GC administration during pregnancy include prophylaxis against refractory nausea and vomiting associated with pregnancy [(10)].

The failure of implantation was attributed to 15% of pregnancy lose [(11,12)].The materno-embryonic contact in the animals, including the rat, which are having haemochorial placentation is usually characterized by appearance of decidual tissues at the interface between the blastocyst and material tissues [(13, 14)] . Blastocyst implantation and successful establishment of pregnancy require delicate interactions between the embryo and the maternal environment [(15 , 16)] . As a matter of fact our original aim of this study was to check if the use of Dx is safe during pregnancy or not? . We have chosen the implantation period, which happening in the rat during the day 6 of pregnancy, because the blastocyst implantation is very critical period of pregnancy.

Materials and Methods.

1. Determination of gestational age:

In this study, fifty five, eight-week-old inbred male and female rats were purchased from the animal house of college of veterinary medicine,AL-Qadysia university. They were maintained under light program of LD 12:12 and fed ad libitum. All experimental rats taken were weighing 175-225 gram. The female rats were mated with male rats and checked for a vaginal plug every morning. The presence of the vaginal plug was considered to indicate day 0 of pregnancy.

2. Animal treatment:

All female rats were divided into two main groups viz: control and treated: The treated group comprised 28 female rats. Pregnant rats were treated daily at day 0 of pregnancy with intraperitoneal injections of dexamethasone17 . They were subdivided into two subgroups in accordance to volume of administered drug. The female rats of the first subgroup (G1)(14 rats) were given intraperitoneally 0.2 mg\kg (of body weight) of dexamethasone. The second subgroup (G2) (14 rats) were given 0.4 mg\kg of dexamethasone. The control group comprised fourteen female rats, have received intraperitoneal injection of same volume of physiological normal saline at day 0 of pregnancy. Half of the pregnant rats of all subgroups were sacrificed on day 7 of pregnancy; the other half was sacrificed on day 10 of pregnancy.

3. Tissue sampling and processing:

Rats from all subgroups in each critical day were anaesthetized with ether, lapromatized; the uteri were then removed and divided into segments containing the implantation sites. These sites were fixed in 10% formalin saline for 48 hours. The samples then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. The blocks were carefully oriented to have the cross sections to be cut from the implantation sites. Five µm thickness serial sections containing the implantation sites only were cut. The sections were deparaffinied and hydrated for haemotoxylin and eosin staining.

Results:

Day 7 dpc (G1 group):

The findings for hematoxylin and eosin stained sections taken from the implantation sites of control and treated rats at this day of pregnancy were showing the same results regarding the arrangement of decidual tissue. The initial site of endometrial stromal cells modification for decidualiation ,which have been considered as an indication of successful implantation, was in the antimesometrial side of endometrium. In the sections stained with hematoxylin and eosin four main zones could be identified (Fig. 1):

1- The primary decidual (PDZ): of closely packed decidual cells that surround the blastocyst and luminal epithelium. (Fig. 1). Neither spaces no blood vessels were detected between the cells of this zone,
2- The secondary decidual zone (SDZ) situated between PDZ and Undifferentiated basal zone (UBZ) and occupying most of the area of endometrium forming a circle around the PDZ. (Fig. 1).There were spaces and blood channels were noticed between the cells of this zone.
3- The implantation zone (IZ) was a small zone located antimesometrial to the embryo where the epithelium was denudated. (Fig. 2)
4- UBZ: It was a narrow band of tissue extended about ¾ of the way around the circumference of the endometrium separating the decidual of SDZ from the inner circular layer of the myometrium. (Fig. 1).

Some sections of decidual tissue were showing evidence of mitotic figures (Fig. 4) among the decidual cells.

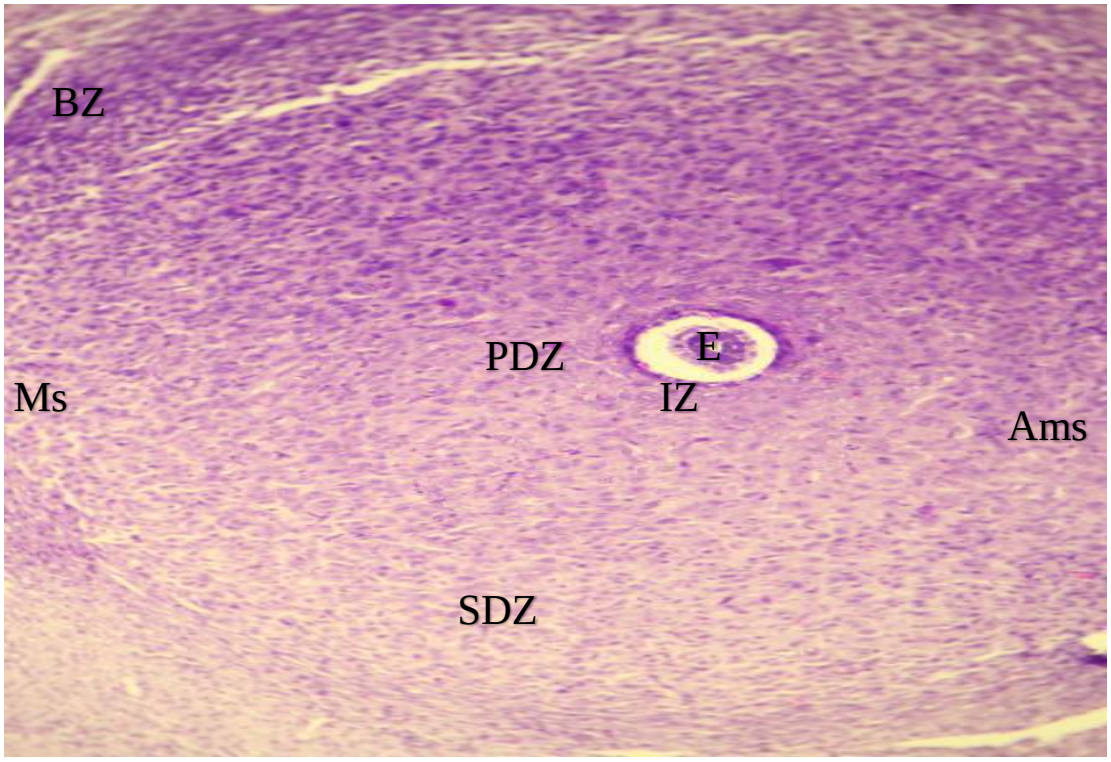

Figure 1- :Cross section in Rat uterus on 7 dpc (Control. subgroup = female rats were orally dosage Normal saline). Note the presence of decidual reaction. PDZ= Primary decidual zone; SDZ=secondary decidual zone; BZ= basal zone, IZ = implantation zone; Ms= mesometrial side of endometrium; Am = antimesometrial side. Hematoxylin and Eosin stain 100X.

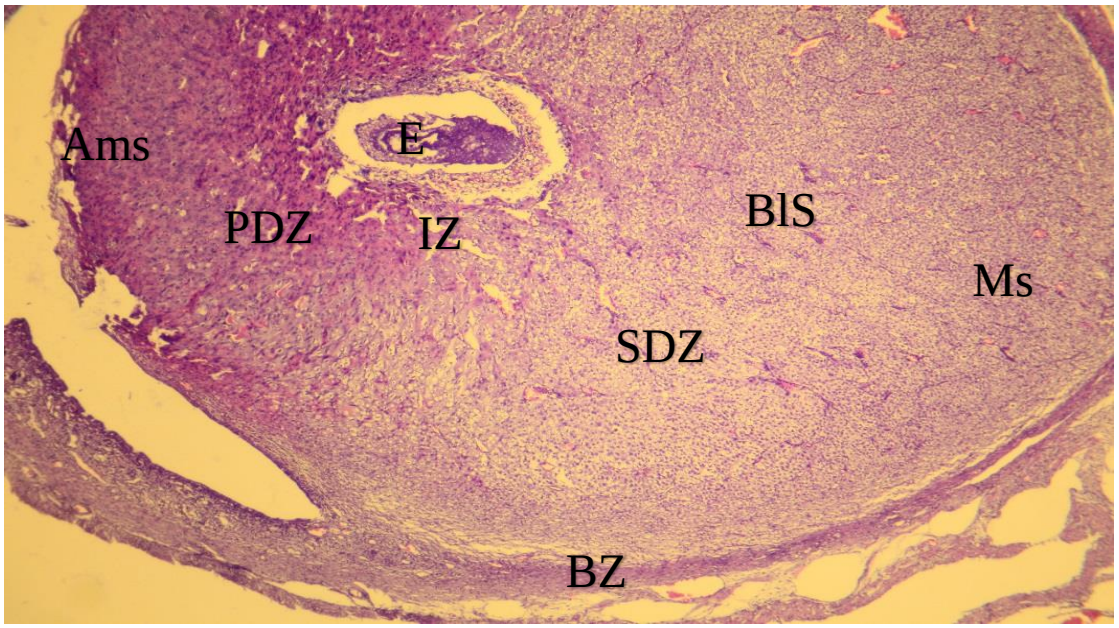

Figure 2 : Cross section in Rat uterus on day 7 dpc(subgroupTh. = female rats were treated with therapeutic dose of the Dexamethasone. Note the presence of decidual reaction. PDZ= Primary decidual zone; SDZ=secondary decidual zone; BZ= basal zone, IZ = implantation zone; Ms= mesometrial side of endometrium; Am = antimesometrial side; BlS= blood sinusoids of the mesometrial decidual zone. Hematoxylin and Eosin stain 40X.

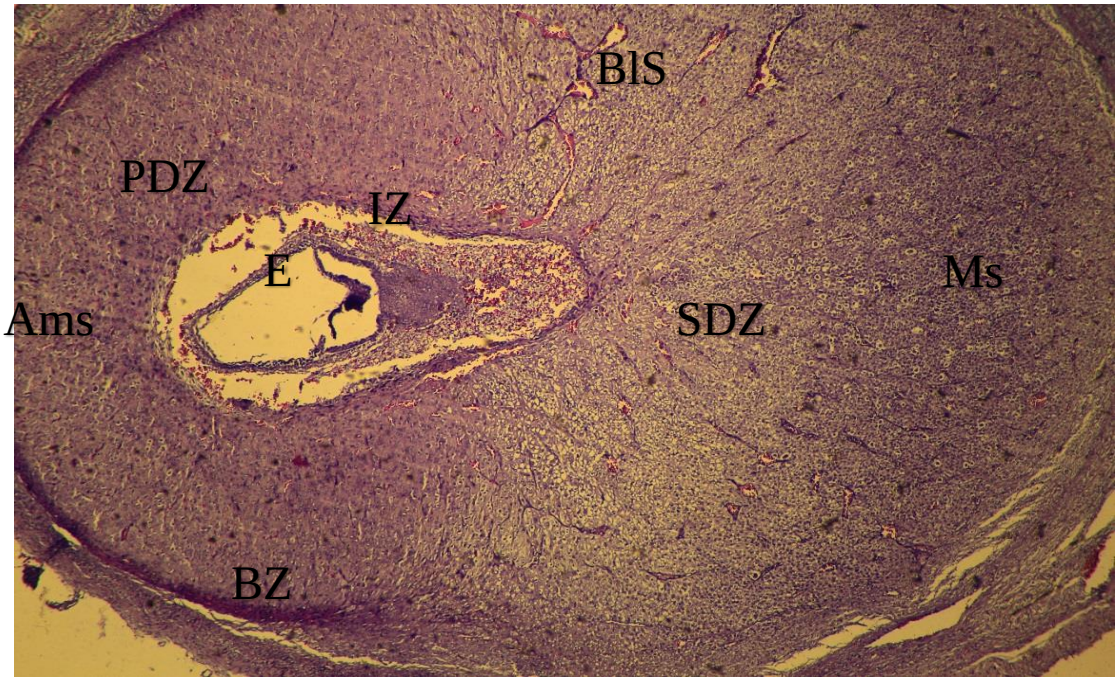

Figure 3 : Cross section in Rat uterus on day 7 dpc. (Therap.subgroup, treated with double therapeutic dose of Dexamethasone) . Note the presence of decidual reaction. PDZ= Primary decidual zone; SDZ=secondary decidual zone; BZ= basal zone, IZ = implantation zone; Ms= mesometrial side of endometrium; Am = antimesometrial side; BlS= blood sinusoids of the mesometrial decidual zone. Hematoxylin and Eosin stain 40X.

Day 10 dpc(G2 group*)*

Grossly the implantation sites at day 10dpc were exhibiting very clear bead-like appearance. The embryo at this stage of pregnancy has shifted from the antimesometrial side to mesometrial side of the uterus. Subsequently decidualization has proceeded mesometrially. Decidualization was in the highest degree of growth and development .In hematoxylin and eosin stained sections, the endometrium could be divided into two main zones (Fig.2):

1. Undifferentiated basal zone (UBZ): A zone of nondecidulized, undifferentiated stromal cells, located close to the myometrium.
   The cells of this zone resembled the fibroblast- like cells of the original endometrium. They have wide extracelullar space.
2. Mesometrial decidual zone (MDZ): The MDZ was occupying a triangular area of endometrium located between the myometrium and the mesometrial pole of embryo .The decidual cells of this zone were not densely packed as in ADZ. There was large tortuous blood spaces associated with this zone radiating from the mesometrial pole of the embryo toward the mesometrial triangle (Fig.5).

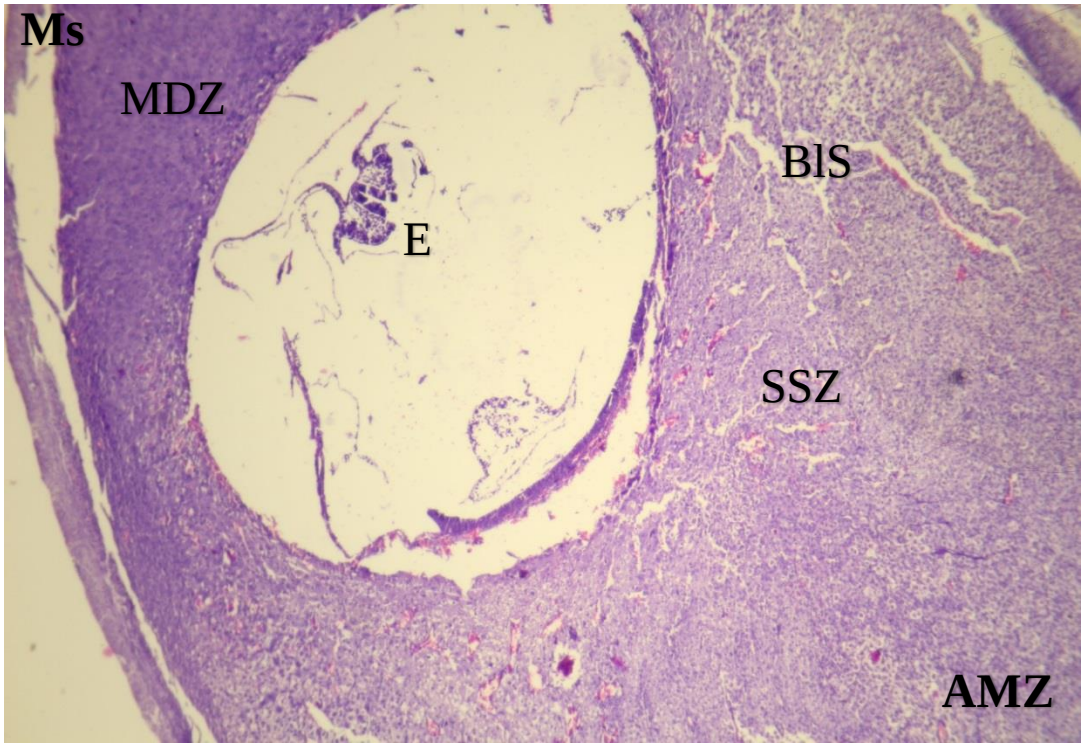

Figure 4 : Day 10 dpc (Control. subgroup = female rats were orally dosage Normal saline).A section of the uterus from rat, passing through the implantation site. Notice the rotation of the embryo (E) mesometrially. Blood sinusoids (BlS) radiating toward the mesometrial side (Ms) where larger blood vessels are there MDZ= mesometrialdecidual zone ; AMZ=Antimesometrial decidual zone; SSZ=spiny shaped decidual zone. Hematoxylin and Eosin stain 40X .

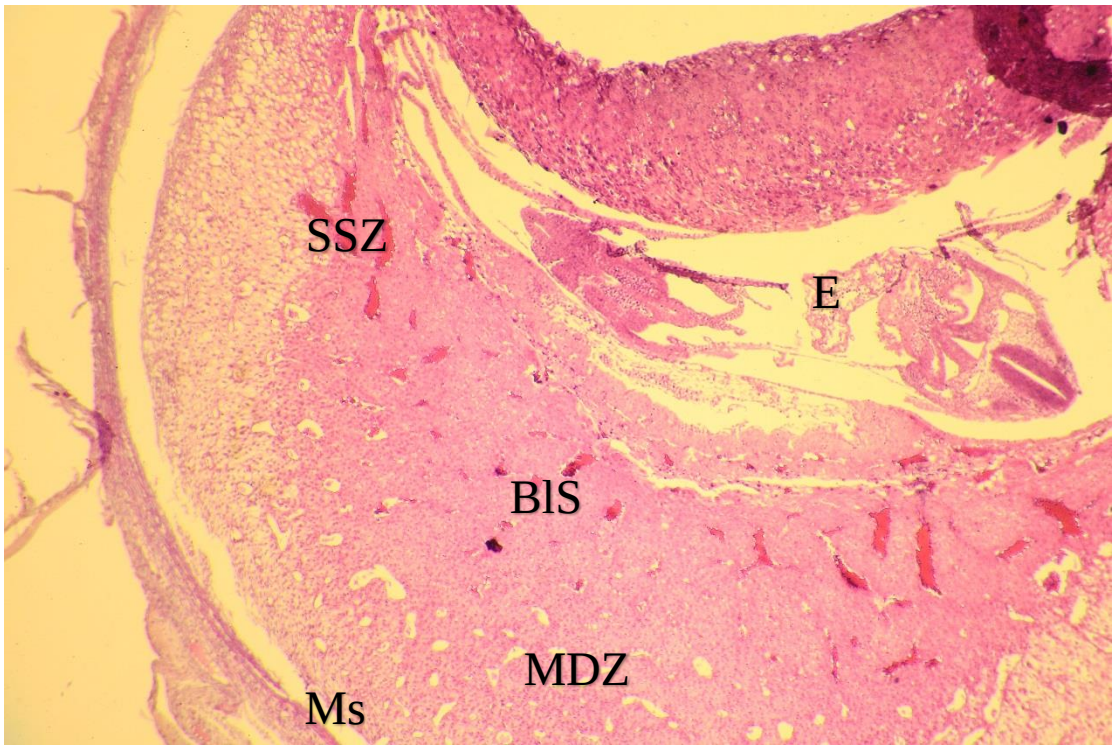

Figure 5 : Day 10 dpc (Therap.Dexamethasone subgroup = female rats were treated with therapeutic dose).A section of the uterus from treated rat, passing through the implantation site. Notice the rotation of the embryo (E) mesometrially. Blood sinusoids (BlS) radiating toward the mesometrial side (Ms) where larger blood vessels arethere.MDZ= mesometrial decidual zone ; SSZ = spiny shaped decidual zone. Hematoxylin and Eosin stain.40X.

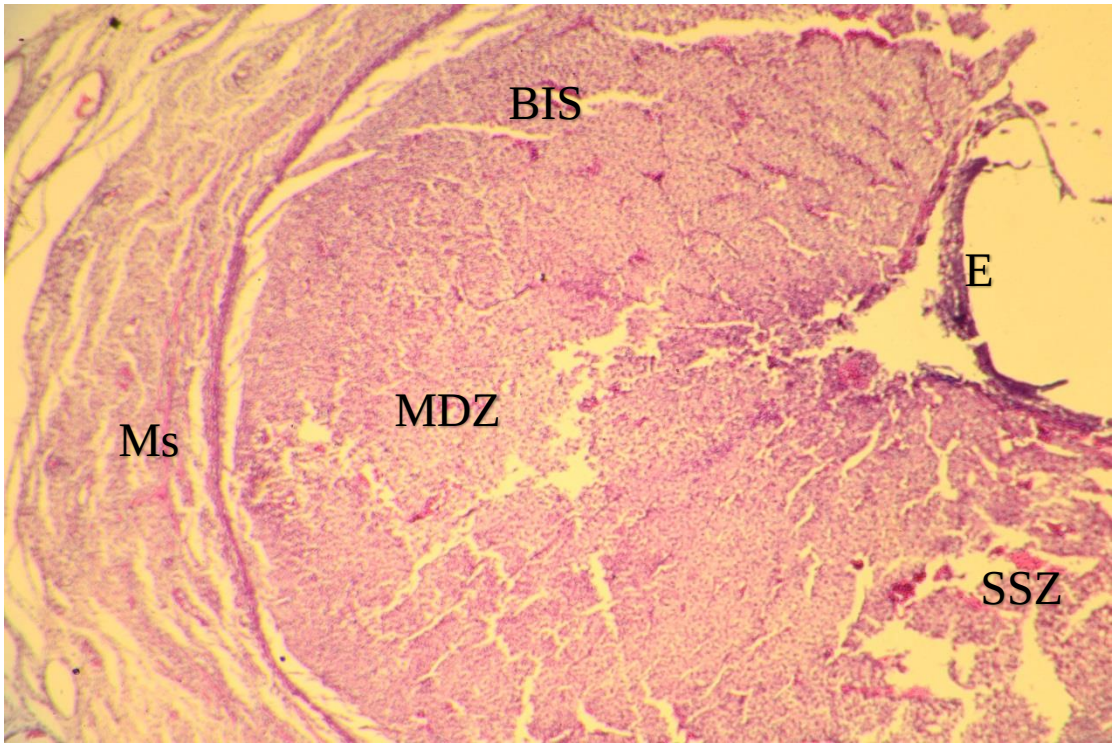

Figure 6 : Day 10 dpc (Therap. Dexamethasone subgroup = female rats were treated with double therapeutic dose).A seaction of the uterus from treated rat, passing through the implantation site. Notice the rotation of the embryo (E) mesometrially. Blood sinusoids (BS) radiating toward the mesometrial side (Ms) where larger blood vessels are there. MDZ= mesometrial decidual zone ; AMZ=Antimesometrial decidual zone ; SSZ =spiny shaped decidual zone. Hematoxylin and Eosin stain 40X.

Discussion

Successful pregnancy requires coordination of three interdependent processes: embryo development, placenta formation, and decidualization of maternal tissue.

One of the events which have been noticed in the present study was the proliferation and differentiation of endometrial cells to be decidual cells in both the control and treated female rats of both the control and treated groups. It has been demonstrated that in early pregnancy the decidual tissue formation, occurring in the endometrium, was leading to a receptive uterus [2,14,15,16,18]. It is often stated that the decidual process is necessary to protect the integrity of the mother against the invading trophoblast [14]. The differentiating stromal cell becomes rounded, acquires myofibroblasts characteristics [16, 18] and secretes a variety of phenotypic antigens, including prolactin, tissue factor, and insulin-like growth factor binding protein-1,34,35 [19,20].

One of the criteria and prerequisite for successful blastocyst implantation in the rat is the appearance of decidual tissues [11, 21] . In fact the presence of decidual tissue is considered as one of the signs of receptivity of the embryo by the uterus [11,14] In the present study the sequence of events of successful implantation was manifested by the appearance of decidual tissues and their zonation on day 7 of pregnancy at the antimesometrial side of the uterus. At day 10 of pregnancy there was shifting of the embryo toward the mesometrial side of the uterus. Several studies have highlighted the importance of the movement of the embryo from the antimesometrial side to the mesometrial side [16]. The mesometrial side is housing anatomically the mesometrial triangle [11] . The importance of the mesometrial triangle for the embryo during the implantation period, which usually span the days 6 to 9 of pregnancy, because it is nearer to the source of blood supply and nutrition from the maternal side which are coming through the uterine arteries and their subsequent branches represented here by the spiral arteries [11] . The appearance of decidual tissue is part of scenario which normally starts with proper growth and development of the uterine endometrium, both spatially and temporally, for blastocyst implantation [14]. Hormonal balancing between the progesterone and estrogen are there for the increase in number of endometrial fibroblast stromal

cells and morphodifferentiation and functional adaptation to be ready for the implanting blastocyst [24].In rat [21] and mice [18] the implanted blastocyst, in both species of animal, is usually trigger the decidual reaction at the antimesometrial side of endometrium ,where the blastocyst is initially attached [21] .Following attachment of blastocyst there will be invasion of trophoblast cells which stimulate stromal fibroblast from the subepithelial side of the antimesometrial endometrium to differentiate and become the primary decidual zone. As mentioned in the present study and previous studies [21,22] the primary decidual zone was characterized by absence of blood vessels and close contact between the decidual cells represented electron microscopically by the presence of tight junctions [21] .Those two features of the primary decidual zone are important to avoid the spread of deleterious materials from the blood circulation to the recently implanted blastocyst .Previous tracer studies using lanthanum nitrate[23] ,injected to the blood circulation, have pointed out the inability of lanthanum nitrate to cross the primary decidual zone . By day 8, the cells comprising the PDZ have progressively degenerated by apoptosis and mostly disappeared [15].

The receptive uterus provides a hospitable environment for blastocyst implantation and the establishment and maintenance of pregnancy [20].

It has been pointed out that dexamethasone and other synthetic glucocorticoids can exert a range of positive effects that would be expected to promote the establishment of early pregnancy, such as suppressing uterine natural killer (uNK) cells and stimulating human chorionic gonadotropin secretion, as well as promoting trophoblast proliferation and invasion [24].

In conclusion, we can say that the using of Dx is safe during early pregnancy according to our stady . However, synthetic glucocorticoids can also exert a range of adverse effects that would be expected to impede pregnancy, induce placental and/or decidual apoptosis, and impair placental nutrient transport including intra-uterine growth restriction, pre-term labour, pre-eclampsia [24].

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